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(71) Applicant: GENTA INCORPORATED [US/US]; 3550 General Atomics Court, San Diego, CA 92121 (US).

(72) Inventors: ARNOLD, Lyle, J., Jr. ; 5439 Noah Way, San Diego, CA 92117 (US). MAXWELL, Kameron ; 16035 Avenida Calma, Rancho Santa Fe, CA 92067 (US).

(74) Agents: BIGGS, Suzanne, L. et al.; 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US).

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(54) Title: COMPOSITIONS AND DELIVERY SYSTEMS FOR TRANSDERMAL ADMINISTRATION OF NEUTRAL OLIGOMERS

(57) Abstract

Neutral Oligomers may be delivered transdermally and across mucous membranes for therapeutic purposes, such as to block expression of a specific target nucleic acid sequence. These neutral Oligomers are useful in treating a variety of pathological conditions, including those of the skin and mucous membranes, as well as conditions effecting other tissues.

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DESCRIPTION

Compositions and Delivery Systems for Transdermal Administration of Neutral Oligomers

Background of the Invention

The use of antisense oligodeoxyribonucleotides for the treatment of conditions caused by viruses such as Herpes viruses, has been proposed. In order for Oligomers to be useful as therapeutic agents, they must be able to gain access to their sites of action. Since most of these sites of action are within the body of the animal to be treated, is necessary that the Oligomers be able to enter the body tissues. Moreover, such Oligomers may need to be able to cross cell membranes and to enter individual cells. It is possible that access to the body be achieved by injection, but this is not a preferred route of access, especially in cases where repeated administration or self-administration is involved. More preferred routes are by either skin or mucous membrane absorption. Mucous membrane absorption can occur within the nasal passages, oral cavity, lung, upper or lower gastrointestinal tract or vaginal tract. Topical skin delivery is also a preferred route of administration. Local delivery of these therapeutic agents has the additional benefit of potentially concentrating these agents near the site of action for enhanced effectiveness of the applied dose. This latter mode of application can also greatly reduce the cost of treatment.

What is then needed are Oligomer compositions that can maximally penetrate skin and mucous membranes. Typical drugs administered transdermally are small molecules having molecular weights less than 400 daltons. Oligomers which are macromolecules having molecular weights far in excess of 400 daltons would not be considered likely candidates for transdermal delivery a priori.

Summary of the Invention

The present invention is directed to a method of delivery of an antisense Oligomer for therapeutic purposes by administration across skin or mucous membranes. Said Oligomers may have therapeutic activity by preventing or interfering with expression of a specific nucleic acid target sequence or the protein product of that sequence. In one aspect, this method comprises the application to skin or mucous membrane of a formulation which comprises a neutral Oligomer which is complementary to and which can bind to or interact with said nucleic acid target sequence. These Oligomers may hybridize to a single-stranded target nucleic acid sequence or alternatively may interact with a double-stranded target sequence to form a triple-stranded complex. (See, e.g., USSN 368,027 "Formation of Triple Helix Complexes of Double Stranded DNA Using Nucleoside Oligomers," the disclosure of which is incorporated herein by reference).

Among other factors, the present invention is based upon our surprising finding that formulations comprising these neutral Oligomers which have no ionic internucleosidyl linkages are able to penetrate skin or mucous membrane at a substantially higher rate than Oligomers which have ionic internucleosidyl linkages. In fact, we have found that Oligomers having only one ionic internucleosidyl linkage and the remainder of the internucleosidyl linkages neutral internucleosidyl linkages penetrate skin at a substantially lower rate than the neutral Oligomers of the present invention.

The delivery of these neutral Oligomers across skin or mucous membranes can be used in the direct or systemic treatment of various conditions by preventing expression of a nucleic acid target sequence. In particular, delivery of these neutral Oligomers across mucous membranes of the nose, the lung or gastrointestinal tract is suitable for systemic treatment, as well as direct treatment of

that tissue. These neutral oligomers may be used in direct treatment of skin or vaginal tissue.

The direct delivery of these neutral Oligomers across skin or mucous membranes may be used to prevent expression of specific nucleic acid sequences which may comprise either foreign nucleic acids from, for example, a virus, or an endogenous nucleic acid sequence. Thus, according to the present invention, topical administration of these neutral Oligomers may be used against viral, fungal and bacterial infections of the skin and mucous membranes such as genital warts caused by the human papilloma virus and infections caused by Herpes viruses. Alternatively, topical administration of neutral Oligomers may be used to block mediators of inflammation. In another aspect, transdermal delivery of these neutral Oligomers may be used to treat conditions in which improper immune or inflammatory responses have been implicated such as psoriasis, atopic dermatitis, eczema, rheumatoid arthritis, allergic rhinitis and the like.

In an additional aspect, transdermal delivery of these neutral oligomers may be used to treat certain cancers of the skin and mucous membranes such as melanoma, mycosis fungoides, and squamous cell carcinoma (including of the cervix) by blocking of the expression of certain cellular factors which are involved in their proliferation.

The delivery of these neutral Oligomers across mucous membranes may be used in systemic treatment of a number of conditions. For example, these neutral Oligomers may be administered by inhalation to nasal and lung mucous membranes. The systemic delivery of these Oligomers may be used to prevent expression of specified nucleic acid target sequences which may comprise either foreign nucleic acid from, for example a virus, or an endogenous nucleic acid sequence. One proposed target is to regulate overexpression of renin in the kidney. Since these Oligomers are reported to be cleared by the body through the kidney

(see Colvin, O.M., et al., Drug Metabolism and Disposition, Vol. 18, pages 815-818 (1990)), administration of Oligomers across mucous membranes should result in a therapeutically effective concentration of Oligomer in the kidney.

In one aspect, the present invention is directed to compositions and delivery systems for the direct or systemic administration of neutral Oligomers across skin or mucous membranes. In one preferred aspect, the compositions of the present invention comprise a neutral Oligomer in a vehicle which comprises a short chain aliphatic alcohol, preferably ethanol. Optionally, the vehicle comprises a flux enhancer which increases transdermal (or transmembrane) flux of said oligomer. Suitable enhancers include compounds which disrupt the skin or mucous membrane barrier to the Oligomer and/or change the partitioning behavior of the Oligomer to the skin or mucous membrane. Suitable enhancers include decylmethylsufoxide, and cyclic ketones, lactones, anhydrides or esters such as those described in PCT application PCT/US86/02583 (Publication No. WO 87/03473), and other enhancers known to those skilled in the art.

According to another aspect of the present invention, compositions for transdermal delivery may optionally include a retention enhancer which increases retention of the Oligomer in the skin, particularly in the viable tissue (rather than the stratum corneum). Suitable retention enhancers include cyclic ketones such as those described in PCT/U586/02583 (Publication No. WO87/03473).

Definitions

As used herein, the following terms have the following meanings, unless expressly stated to the contrary:

The term "nucleoside" includes a nucleosidyl unit and is used interchangeably therewith, and refers to a subunit of a nucleic acid which comprises a 5 carbon sugar and a nitrogen-containing base. In RNA the 5 carbon sugar is

ribose; in DNA, it is a 2'-deoxyribose. The term also includes analogs of such subunits.

A "non-nucleoside monomeric unit" refers to a monomeric unit which does not significantly participate in hybridization of an Oligomer to a target sequence. Such monomeric units must not, for example, participate in any significant hyhdrogen bonding with a nucleoside, and would exclude monomeric units having as a component, one of the 5 nucleotide bases or analogs thereof.

A "nucleoside/non-nucleoside polymer" refers to a polymer comprised of nucleoside and non-nucleoside monomeric units.

The term "oligonucleoside" or "Oligomer" refers to a chain of nucleosides which are linked by internucleoside linkages which is generally from about 6 to about 100 nucleosides in length, but which may be greater than about 100 nucleosides in length. They are usually synthesized from nucleoside monomers, but may also be obtained by enzymatic means. Thus, the term Oligomer refers to a chain of oligonucleosides which have internucleosidyl linkages linking the nucleoside monomers and thus, includes oligonucleotides, nonionic oligonucleoside alkyl and aryl-phosphonate analogs, alkyl- and aryl-phosphonothioates phosphorothioate analogs of oligonucleotides, phosphoramidate analogs of oligonucleotides, neutral phosphate ester oligonucleoside analogs, such as phosphotriesters and other oligonucleoside analogs and modified oligonucleosides, and also includes nucleoside/non-nucleoside polymers. The term also includes nucleoside/non-nucleoside polymers wherein one or more of the phosphorous group linkages between monomeric units has been replaced by a non-phosphorous linkage such as a, morpholino linkage, a formacetal linkage, a sulfamate linkage or a carbamate linkage.

The term "alkyl- or aryl-phosphonate Oligomer" refers to Oligomers having at least one alkyl- or aryl-phosphonate internucleosidyl linkage.

The term "methylphosphonate Oligomer" (or "MP-Oligomer") refers to Oligomers having at least one methylphosphonate internucleosidyl linkage.

The term "neutral Oligomer" refers to Oligomers which have nonionic internucleosidyl linkages between nucleoside monomers (i.e. linkages having no net positive or negative ionic charge) and include, for example, Oligomers having internucleosidyl linkages such as alkyl- or aryl-phosphonate linkages, alkyl- or aryl-phosphonothioates, neutral phosphate ester linkages such as phosphotriester linkages, especially neutral ethyltriester linkages; and non-phosphorus-containing internucleosidyl linkages, such as sulfamate, morpholino, formacetal and carbamate linkages. Optionally, a neutral Oligomer may comprise a conjugate between a oligonucleoside or nucleoside/non-nucleoside polymer and a second molecule which comprises a conjugation partner. Such conjugation partners may comprise intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents, photo-cross-linking agents such as psoralen, and the like. Such conjugation partners may further enhance the uptake of the Oligomer, modify the interaction of the Oligomer with the target sequence, or alter the pharmacokinetic distribution of the Oligonucleoside. The essential requirement is that the oligonucleoside or nucleoside/non-nucleoside polymer that the conjugate comprises be neutral.

The term "neutral alkyl- or aryl- phosphonate oligomer" refers to neutral oligomers having neutral internucleosidyl linkages which comprise at least one alkyl- or aryl- phosphonate linkage.

The term "neutral methylphosphonate oligomer" refers to neutral oligomers having internucleosidyl linkages which comprise at least one methylphosphonate linkage.

In some of the various Oligomer sequences listed herein "p" in, e.g., as in ApA represents a phosphate diester linkage, and "p" in, e.g., as in CpG represents a methylphosphate linkage.

The term "tandem oligonucleotide" or "tandem Oligomer" refers to an oligonucleotide or oligomer which is complementary to a sequence located either on the 5' or 3' side of a target nucleic acid sequence and which is co-hybridized with a second oligomer which is complementary to the target sequence. Tandem oligomers may improve hybridization of these oligomers to the target by helping to make the target sequence more accessible to such oligomers, such as by decreasing the secondary structure of the target nucleic acid sequence. In addition, one of a pair of tandem Oligomers may improve the hybrid stability of the second tandem Oligomer by promoting a helical structure at either the 5'- or 3'-end of said second Oligomer and vice-versa.

The term "short chain aliphatic alcohol" refers to an alcohol having from about 2 to about 20 carbon atoms in which the aliphatic (alkyl) chain may be either straight chained or branch chained and includes primary, secondary and tertiary alcohols, glycols and polyols.

The term "flux enhancer" refers to a substance which is used to increase transdermal flux of a compound. A flux enhancer is typically applied to skin or mucous membrane in combination with the compound to increase transdermal flux of the compound. Enhancers are believed to function by disrupting the skin or mucous membrane barrier or by changing the partitioning behavior of the drug in the skin or mucous membrane.

Brief Description of the Drawings

FIG. 1 depicts a plot of cumulative amount of Oligomer penetrating hairless mouse skin versus time for several concentrations formulations of 14-mer in vehicle ($EtOH/H_2O/DMS$, 80:15:5).

FIG. 2 depicts a plot of cumulative amount of a 14-mer having one internal anionic internucleosidyl linkage ("14-mer-1A") penetrating hairless mouse skin versus time (vehicle was EtOH (DMS/95:5).

FIG. 3 depicts a plot of percentage of applied dose of Oligomer absorbed versus time for formulations having varying concentrations of 14-mer.

FIG. 4 depicts a plot of cumulative amounts of 14-mer penetrating human cadaver skin versus times for several formulations of 14-mer.

Detailed Description of the Invention

Preferred Neutral Oligomer Formulations

Preferred neutral Oligomers include neutral alkyl- and aryl- phosphonate Oligomers and neutral Oligomers comprising triester or phosphoramidate internucleosidyl linkages. Especially preferred are neutral methylphosphonate Oligomers. In view of their demonstrated ability to penetrate skin, including tape stripped skin (which has had the stratum corneum removed and which has been reported as a model for mucous membrane), particularly preferred are neutral methylphosphonate Oligomers having only methylphosphonate internucleosidyl linkages.

Synthetic methods for preparing methylphosphonate Oligomers are described in Example 1 herein and also in Lee B.L., et al., Biochemistry 27:3197-3203 (1988), and Miller, P.S., et al., Biochemistry 25:5092-5097 (1986), the disclosures of which are incorporated herein by reference.

According to another aspect of the present invention, preferred are Oligomers which comprise one or more triester internucleoside linkages, especially neutral ethyltriester internucleoside linkages. It is believed that once such Oligomers have entered the cell, the ester group is cleaved to give a negatively charged diester linkage which is better retained inside the cell. According to one preferred embodiment, such Oligomers may incorporate either one or more methylphosphonate internucleoside linkages or one or more nucleosides having a 2'-O-methyl ribosyl moiety. The presence of methylphosphonate internucleoside linkages at the ends of the Oligomer gives

exonuclease resistance and the incorporation of methylphosphonate internucleoside linkages or nucleosides which comprise a 2'-O-methyl ribosyl moiety in the middle of the Oligomer increases resistance of the Oligomer to endonucleases.

Preferred are neutral Oligomers having from about 6 to about 40 nucleosides, more preferably from about 12 to about 20 nucleosides. Although neutral Oligomers which comprise more than 20 nucleosides may be used, where complementarity to a longer sequence is desired, it may be advantageous to employ shorter tandem neutral Oligomers to maximize solubility and penetration through the skin or mucous membranes while competing for the development of a secondary structure of the target nucleic acid, such as a mRNA. Alternatively, it may be advantageous to use more than one neutral Oligomer, each Oligomer complementary to a distinct target sequence which may be part of the same gene or a different gene.

Where the neutral Oligomers comprise alkyl- or arylphosphonate Oligomers, it may be advantageous to incorporate nucleoside monomeric units having modified ribosyl moieties. The use of nucleoside units having 2'-O-alkyl- and, in particular, 2-O-methyl-ribosyl moieties in these neutral Oligomers may advantageously improve hybridization of the Oligomer to its complementary target sequence.

According to one preferred aspect, these neutral Oligomers may comprise a conjugate between a polynucleoside or nucleoside/non-nucleoside polymer and a conjugation partner. Suitable conjugation partners include intercalating agents, alkylating agents, binding substances for cell surface receptors, lipophilic agents, photo-crosslinking agents such as psoralen, hydrolytic or nucleolytic agents, pro-chelates or DNA modifying agents.

These conjugation partners may include groups such as photo-crosslinking groups like psoralen, cross-linking agents, intercalating agents such as acridine, or groups capable of cleaving a targeted portion of a nucleic acid

such as hydrolytic or nucleolytic agents like o-phenanthrolinecopper or EDTA-iron may be incorporated in the Oligomers.

These Oligomers may be labelled by any of several well known methods. Useful labels include radioisotopes. Isotopic labels include ^{3}H, $^{35}5$, ^{32}P, ^{125}I, Cobalt and ^{14}C. Most methods of isotopic labelling involve the use of enzymes and include the known methods of nick translation, and labelling, second strand synthesis, and reverse transcription.

Conjugation partners may also be introduced into the Oligomer by the incorporation of modified nucleosides or nucleoside analogs through the use of enzymes or by chemical modification of the Oligomer, for example, by the use of nonnucleotide linker groups.

When used to prevent function or expression of a single or double stranded nucleic acid sequence, these Oligomers may be advantageously derivatized or modified to incorporated a nucleic acid modifying group which may be caused to react with said nucleic acid and irreversibly modify its structure, thereby rendering it non-functional.

Commonly assigned USSN 565,299, the disclosure of which is incorporated herein by reference, discloses psoralen-derivatized Oligomers.

A wide variety of DNA modifying groups may be used as conjugation partners to derivatize these Oligomers. DNA modifying groups include groups which, after the derivatized Oligomer forms a complex with a single stranded or double stranded DNA segment, may be caused to form a covalent linkage, cross-link, alkylate, cleave, degrade, or otherwise inactivate or destroy the DNA segment or a target sequence portion thereof, and thereby irreversibly inhibit the function and/or expression of that DNA segment.

The location of the DNA modifying groups in the Oligomer may be varied and may depend on the particular DNA modifying group employed and the targeted double stranded

DNA segment. Accordingly, the DNA modifying group may be positioned at the end of the Oligomer or intermediate the ends. A plurality of DNA modifying groups may be included.

In one preferred aspect, the DNA modifying group is photoreactable (e.g., activated by a particular wavelength, or range of wavelengths of light), so as to cause reaction and, thus, cross-linking between the Oligomer and the double stranded DNA.

Exemplary of DNA modifying groups which may cause cross-linking are the psoralens, such as an aminomethyl-trimethyl psoralen group (AMT). The AMT is advantageously photoreactable, and thus must be activated by exposure to particular wavelength light before cross-linking is effectuated. Other cross-linking groups which may or may not be photoreactable may be used to derivatize these Oligomers.

Alternatively, the DNA modifying groups may comprise an alkylating agent group which is covalently bonded to the DNA segment to render it inactive. Suitable alkylating agent groups are known in the chemical art sand include groups derived from alkyl halides, haloacetamides, and the like.

DNA modifying groups which may be caused to cleave the DNA segment include transition metal chelating complexes such as ethylene diamine tetraacetate (EDTA) or a neutral derivative thereof. Other groups which may be used to effect cleaving include phenanthroline, porphyrin and the like. When EDTA is used, iron may be advantageously tethered to the Oligomer to help generate the cleaving radicals. Although EDTA is a preferred DNA cleaving group, other nitrogen containing materials, such as azo compounds or nitrenes or other transition metal chelating complexes may be used.

Suitable formulations comprise about 0.0001% to about 2% by weight of neutral Oligomer.

In one preferred aspect, these neutral Oligomer formulations comprises about 2% to about 100% of a short chain aliphatic alcohol. Suitable alcohols include ethanol, isopropyl alcohol, propylene glycol and glycerol. In certain studies, formulations of neutral Oligomers comprising ethanol have demonstrated advantageous transdermal flux.

In an especially preferred aspect, these neutral Oligomer formulations may additionally comprise a flux enhancer. Suitable flux enhancers include those known to those skilled in the art and include decylmethylsulfoxide, dimethylsulfoxide as well as cyclic ketones, lactones, anhydrides and esters such as those disclosed in PCT Application No. PCT/US86/02583 (Publication Number WO87/03473). Some of these flux enhancers also increase retention of the Oligomer and, thus, act to increase the concentration of Oligomer within the skin itself.

Thus, for Oligomer formulations for direct (local) treatment, such as topical application to skin, it is preferred to use a flux enhancer which not only maximizes transdermal flux, but increases Oligomer retention in the skin. Certain cyclic ketone and lactone enhancers have been reported to increase local retention as well and, thus, comprise a preferred class of enhancers for topical administration of Oligomer formulations.

In Oligomer formulations for systemic treatment, it is preferable to use a flux enhancer which maximizes flux with a minimal increase of local retention of Oligomer.

Preferred Targets and Target Sequences

According to one aspect, the present invention provides methods of preventing or interfering with expression of a specific target nucleic acid sequence by the transdermal administration of a neutral Oligomer which is complementary to and which can bind to or interact with a specific target nucleic sequence. These neutral Oligomers can penetrate skin or mucous membrane tissue. These neu-

tral Oligomers may be applied to skin or mucous membrane to treat a variety of conditions either locally in the skin or mucous membrane (such as lung, gastrointestinal tract or vaginal tissue) or systemically by blocking or interfering with the expression of a specific target nucleic acid sequence. The sequence to be blocked may comprise a "foreign" target nucleic acid sequence (i.e., one from an outside source such as a pathogen) or one of endogenous origin.

Thus, in one aspect these neutral Oligomers may be used to directly treat viral, bacterial, fungal and other infections of the skin or mucous membranes caused by pathogens. Such conditions include those caused by a Herpes virus, a human papilloma virus (such as genital warts), or species of Pseudomonas, Staphylococcus or Helicobacter.

These neutral Oligomers may also be used to block mediators of inflammation. These mediators include cytokines, growth factors, cell adhesion molecules or their ligands and receptors thereof as well as key enzymes in pathways leading to inflammation. These blocking actions include preventing the expression of cytokines (such as IL-1), growth factors (such as TGF-α and EGF), or cell adhesion molecules (such as ELAM and ICAM); or the receptors for cytokines (such as IL-1), growth factors, or cell adhesion molecules. Key enzymes whose expression may be blocked include protein kinase C and phospholipase A or C. Neutral Oligomers which block the expression of phospholipase A_2 (PLA_2) or the receptor for IL-1 may be useful to treat inflammation of joints due to rheumatoid arthritis.

These neutral Oligomers may be used therapeutically to treat immune or inflammatory-response related diseases or conditions. Neutral Oligomers which block the expression of IL-1, Transforming Growth Factor α (TGFα), amphiregulin, or IL-6 may be useful in treating psoriasis. These neutral Oligomers may also be useful in the treatment of atopic dermatitis. Furthermore, these Oligomers

may be used to block overexpression of IgE, said to be mediated by mast cells releasing histamine, and to be useful in the treatment of atopic dermatitis, eczema and asthma. Oligomers which block IL-4 expression may be useful in the treatment of allergic rhinitis.

Additionally, these Oligomers may be used in therapy of certain skin-related cancers such as melanoma and squamous cell carcinoma, by blocking the expression of certain factors which promote cell growth and/or adhesion and are believed to be involved in metastasis. These factors include Epidermal Growth Factor (EGF), and certain cellular adhesion factors.

These neutral Oligomers may be used to prevent expression of mediators of cell adhesion or their receptors so there will be no focus for cell recruitment or cell adhesion at a point of injury. These mediators include ELAM, ICAM, and thrombospondin. These Oligomers may also be used to block expression of retinoic acid receptors.

These Oligomers may be useful to treat septic shock by blocking the expression of the IL-1 receptor antagonist.

In general, preferred are target nucleic acid sequences which comprise a region of the gene to be blocked that is required for its expression. These target sequences may comprise a portion of the gene, or a portion of a mRNA transcript thereof which is "available", i.e., is in a state where the complementary neutral Oligomer is able to hybridize to the target sequence. Thus, these target sequences are preferably single stranded and relatively free of secondary structure and bound protein.

Suitable target sequences include sequences which are at or proximate to a 5'-terminal translational start, a 3'-terminal polyadenylation signal, a mRNA cap site or a splice junction.

According to one preferred aspect of the present invention, expression of renin, especially in the juxtaglomerular apparatus (JGA), is blocked by administration

of these neutral Oligomers to skin or mucous membranes. Thus, a preferred target sequence for the neutral Oligomers of the present invention comprises a sequence within the renin gene or its mRNA transcript. Renin is an enzyme which catalyzes the cleavage of angiotensinogen to produce angiotensin I. Angiotensin I is further cleaved by angiotensin converting enzyme to angiotensin II, which is a potent extracellular messenger involved in vasoconstriction. Angiotension II also stimulates the adrenal cortex to secrete aldosterone. Neutral Oligomers which are complementary to targets in the renin gene or mRNA transcripts thereof may be preferably administered by inhalation to the mucous membrane of the lung, thereby passing into the bloodstream, and reaching the kidney where renin is produced. Heretofore, it has been difficult to inhibit renin without side effects.

These neutral Oligomers may also be used to treat chronic myelogenous leukemia by preventing expression of $P210^{bcr/abl}$. (See the commonly assigned, co-pending patent application, USSN 565,299 "Psoralen-Conjugated Methylphosphonate Oligonucleotides as Therapeutic Agents for Chronic Myelogeneous Leukemia", the disclosure of which is incorporated herein by reference.)

Permeability of Oligomer Formulations

The ability of a neutral methylphosphonate Oligomer having 14 nucleosides and having only methylphosphonate internucleosidyl linkages (referred to as "14-mer" herein) to penetrate hairless mouse skin was measured using several concentrations of 14-mer and using several vehicle formulations. (See Table I.)

In comparison, the ability of a second Oligomer (referred to as "14-mer-IA" herein) which was a methylphosphonate Oligomer which contained an internal anionic internucleosidyl linkage (a phosphodiester linkage) to penetrate hairless mouse skin was measured using several vehicle formulations. (See Table I.) There was no mea-

surable amount of 14-mer-IA detected in the receptor solution over the 24 hour test period with either the water or ethanol vehicle formulations, in contrast to the 14-mer in the same vehicle formulations. For the ethanol/decylmethylsulfoxide (95:5) vehicle formulation, the permeability of hairless mouse skin for 14-mer-IA was about 10-times less than for 14-mer; the total amount of 14-mer which penetrated after 24 hours was about 5.5 $\mu g/cm^2$ in comparison to about 0.6 $\mu g/cm^2$ of 14-mer-IA. Therefore, the introduction of just one ionic (negative) internucleosidyl linkage into the neutral Oligomer was demonstrated to significantly decrease the ability of the Oligomer to penetrate skin when compared to the neutral Oligomer. See also Figures 1 and 2 which depict cumulative amount of 14-mer and 14-mer-IA, respectively, which penetrates hairless mouse skin over time.

The stratum corneum is the primary barrier to absorption through the skin for most drugs, including macromolecules. In order to determine the degree to which the stratum corneum limits the absorption of methylphosphonate Oligomers, an experiment was performed wherein the stratum corneum was removed from the skin by tape stripping. Also, tape stripped skin has been reported to have similar permeability characteristics to mucous membrane and, thus, has been proposed as a model system for mucous membrane. Penetration of the 14-mer through normal (unstripped) hairless mouse skin and skin following removal of the stratum corneum was examined and the rates compared. (See Table I.)

Table I gives the cumulative amount of neutral methylphosphonate 14-mer penetrating stripped skins from two vehicles: EtOH/decylmethyl sulfoxide (DMS) (95:5, v/v) and ethanol (EtOH). The amount of the neutral methylphosphonate 14-mer penetrating skin was increased dramatically when the stratum corneum was removed by tape stripping. Removal of the stratum corneum by tape stripping resulted in absorption of the entire dose of 14-mer

absorbed through the stripped skin from both vehicles tested by 24 hours.

An experiment was performed to investigate the effect of changing the concentration of the neutral methylphosphonate 14-mer in the vehicle. Because the EtOH-based vehicles, even with small amounts of a cosolvent such as DMS added, were poor solvents for the neutral methylphosphonate 14-mer, water was added to the vehicle. The vehicle tested was EtOH/H_2O/DMS (80:15:05) in which neutral methylphosphonate 14-mer was added to saturation (3.8 mg/mL), or in concentrations of 0.5 mg/mL, or 0.05 mg/mL. The skin used in these experiments was full-thickness hairless mouse. The cumulative amount of 14-mer penetrating the skin over 24 hours is shown in Figure 1. The concentration of neutral methylphosphonate 14-mer in the vehicle was related to the amount of drug penetrating a unit area of skin. The driving force is related to the concentration of the neutral methylphosphonate 14-mer in the vehicle.

Table I depicts the penetration of 14-mer through hairless mouse skin using several different vehicles and at several different concentrations of 14-mer.

The cumulative amount of 14-mer which absorbed from a test vehicle over time is depicted in Figure 1. The percent of the applied dose absorbed from the test vehicles for those concentrations of 14-mer is shown in Figure 3. The 0.05 mg/mL and 0.5 mg/mL formulations were both solutions, while the saturated (3.8 mg/mL) formulation was a suspension. The sparing solubility of the 14-mer in the vehicle may effect the rate at which it was absorbed.

The cumulative amount of neutral methylphosphonate 14-mer which penetrated after 24 hours from the EtOH/H_2O/DMS (80:15:5) vehicle saturated with drug was about 3.5 $\mu g/cm^2$. This was less than the cumulative amount which penetrated per cm^2 after 24 hours from the EtOH/DMS (95:5) vehicle (saturated) (wherein 5.5 $\mu g/cm^2$ was found to pene-

trate). (See Table I.) The flux using the EtOH/H_2O/DMS vehicle may have been reduced because the solubility of 14-mer in that vehicle was higher than EtOH/DMS and, thus, there was less driving force to partition the 14-mer out of the vehicle into the skin.

The permeability of human skin to the 14-mer was studied using the EtOH/H_2O/DMS (80:15:5) vehicle saturated with neutral methylphosphonate 14-mer. The results of this experiment are depicted in Figure 4 along with the data from the same experiment performed with the EtOH/DMS (95:5) and EtOH vehicles. While the permeability of the human skin toward the 14-mer from the EtOH/H_2O/DMS (80:15:5) vehicle was lower than that from the water-free vehicles, the permeability profile was very similar. Compared to the hairless mouse data, the differences in permeability were minimal. As had been the case with all the vehicles tested with human skin, human skin was much less sensitive to changes in vehicle composition than was hairless mouse skin.

Retention of Oligomer in the Skin

Drug products for topical application to skin should maximize the amount of drug retained in the target area within the skin. In the case of these neutral oligomers, for many of their intended targets, the basal cells of the dermis are the regions to which the drug should be delivered. Permeability experiments may indicate whether drug is being delivered through the skin and, therefore, that drug is being delivered to the dermal layer. However, it is important to know how much drug is retained in the skin at various times during the permeation experiment. Therefore, the skins used in the permeability experiments were routinely collected to analyze the amount of drug retained after 24 hours of exposure to the drug-containing vehicle.

There are several ways that have been reported to assess drug retention. One method has been to homogenize a section of the whole skin used in the permeability

experiments and measure the amount of drug (i.e. oligomer) released from the sample. This technique detects the amount of drug in the entire skin sample (an average of the various strata). A second approach that can be used is the separation of skin into layers (stratum corneum, viable epidermis, and dermis). A number of techniques have been used to separate skin into its component layers. Most techniques suffer from one or more problems, such as leaching of the drug into water during the separation process. For instance, a heat treatment technique for separation of the epidermis and the dermis requires submersion of the skin into hot water for several minutes prior to separation of the layers. To avoid this problem, a microwave technique to separate the dermis and epidermis has been described (Kumar, S., et al., Pharm. Res. 6:740-741 (1989)).

The first type of experiments used to determine drug Oligomer retention were those in which the entire skin sample was examined for Oligomer. The experimental technique used to measure retention is described in Example 5. In Table II, the amounts of 14-mer recovered from skin samples following the 24 hour permeability experiments are listed.

The stratum corneum was removed from whole skin samples using microwave exposure for 5-8 seconds. The amounts of 14-mer recovered from the samples is shown in Table IIB. Both hairless mouse and human skin were used; two samples of each were examined for total tissue retention. Following removal of the stratum corneum from two additional skin samples, the 14-mer content was measured in the remaining viable epidermis and dermis of the samples. Differences were observed between the hairless mouse and human skin data.

To assist in understanding the present invention, the following examples are included which describe the results of a series of experiments. The following examples relating to this invention should not, of course, be construed

in specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the present invention as hereinafter claimed.

Examples

Example 1

Preparation of Methylphosphonate Oligomers

Neutral methylphosphonate oligomers are synthesized using methylphosphonamidite monomers, according to the chemical methods described by P.S. Miller et al. (Nucleic Acids Res. 11:6225-6242 (1983)), A. Jager and J. Engels (Tetrahedron Letters 25:1437-1440 (1984)) and M.A. Dorman et al. (Tetrahedron Letters 40:95-102 (1984)). Solid phase synthesis is performed on a Biosearch Model 8750 DNA synthesizer according to the manufacturer's recommendations with the following modifications: "G" and "C" monomers are dissolved in 1:1 acetonitrile/dichloromethane at a concentration of 100 mM. "A" and "T" monomers are dissolved in acetonitrile at a concentration of 100 mM. DEBLOCK reagent = 2.5% dichloroacetic acid in dichloromethane. OXIDIZER reagent 25 g/L iodine in 2.5% water, 25% 2,6-lutidine, 72.5% tetrahydrofuran. CAP A = 10% acetic anhydride in acetonitrile. CAP B =).625% N,N-dimethylaminopyridine in pyridine. The 5'-dimethoxytrityl protecting group is left on at the end of the synthesis to facilitate purification of the oligomers, as described below.

The crude, protected methylphosphonate oligomers are removed from the solid support by mixing with concentrated ammonium hydroxide for two hours at room temperature. The solution is drained from the support using an Econo-Column™ (Bio-Rad, Richmond, CA) and the support is washed five times with 1:1 acetonitrile/water. The eluted oligomer is evaporated to dryness under vacuum at room temperature. Next, the protecting groups are removed from the

bases with a solution of ethylenediamine/ethanol/acetonitrile/water (50:23.5:23.5:2.5) for 6 hours at room temperature. The resulting solutions are then evaporated to dryness under a vacuum.

The 5'-dimethoxytrityl ("trityl") containing oligomers are purified from non-tritylated failure sequences using a Sep-Pak™ C^{18} cartridge (Millipore/Waters Bedford, MA) as follows: The cartridge is washed with acetonitrile, 50% acetonitrile in 100 mM, triethylammonium bicarbonate (TEAB, pH 7.5) and 25 mM TEAB. Next, the crude methylphosphonate oligomer is dissolved in a small volume of 1:1 acetonitrile/water and then diluted with 25 mM TEAB to a final concentration of 5% acetonitrile. This solution is then passed through the cartridge. Next, the cartridge is washed with 15-20% acetonitrile in 25 mM TEAB to eluate failure sequences from the cartridge. The trityl-on oligomer remaining bound to the cartridge is then detritylated by washing with 25 mM TEAB, 2% trifluoroacetic acid, and 25 mM TEAB, in that order. Finally, the trityl-selected oligomer is eluted from the cartridge with 50% acetonitrile/water and evaporated to dryness under vacuum at room temperature.

The methylphosphonate oligomers are further purified by reverse-phase HPLC chromatography as follows: A Beckman System Gold HPLC is used with a Hamilton PRP-1 column (Reno, NV, 10 μ, 7 mm i.d. x 305 mm long). Buffer A = 50 mM triethylammonium acetate (pH 7); Buffer B = 50% acetonitrile in 50 mM triethylammonium acetate (pH 7). The sample, dissolved in a small volume of 10-50% acetonitrile/water, is loaded onto the column while flowing at 2.5-3 ml/minute with 100-% Buffer A. Next, a linear gradient of 0-70% Buffer B is run over about 30-50 minutes at a flow rate of about 2.5 to 3.0 ml/minute. Fractions containing full length methylphosphonate oligomer are collected, evaporated under vacuum and resuspended in 50% acetonitrile/water.

Example 2

Preparation of Skin Samples for Permeability and Tissue Level Studies

A. Hairless Mouse Skin

The hairless mice (male, HRS/J strain, 8 to 10 weeks old, 20 to 25 g) were sacrificed in a CO_2 chamber and approximately 5 cm^2 of full-thickness skin (dermis and epidermis) was removed from the abdomen. After removal of the subcutaneous fat, the skins were rinsed with physiological saline and used within one hour.

The stratum corneum was removed from hairless mice for permeability experiments by using cellophane tape. The tape was gently applied to the skin of a recently sacrificed animal and then pulled away from the body. This was repeated 12 to 15 times with fresh pieces of tape.

B. Human Cadaver Skin

Human cadaver skin was obtained at autopsy through the Stanford University Medical Center. The skin was excised using a dermatome from the thigh area of a 74 year old male within 24 hours post-mortem. The thickness, as measured with a Van Keuren light wave micrometer, ranged from 125 to 450 μm. The average thickness was 200 to 300 μm. The skin was rinsed with phosphate buffered saline (pH 7.4), blotted dry and frozen for 6 months in triple-sealed bags evacuated of air. Prior to use, the skin was thawed and rinsed in PBS.

Example 3

Permeability Experiments

A diffusion console containing nine glass Franz diffusion cells was used in the permeability experiments. The Franz cells were maintained at 37°C by thermostatically controlled water, which was circulated through a jacket surrounding the cell body. Each skin was mounted and clamped between the cell body and the cell cap so that

the epidermal side faced upward (vehicle side). The skins were then allowed to equilibrate for 1 hour in the diffusion cells prior to addition of the vehicle. The exposed surface was 2.0 cm^2. The receptor was 0.01 M phosphate-buffered saline (pH 6.4) isotonic saline with 0.05% sodium azide added to prevent growth of microorganisms.

The Franz cells were closed to maximize drug concentration in the receptor phase. The volume of the cells was 6.2 mL. the cells were stirred using a teflon-coated stir bar at 600 rpm.

The drug/vehicle mixtures were pipetted through the cell cap on to the skin [0.2 mL total vehicle added to 2.0 cm^2 (0.1 $nK.cm^2$)]. At certain times following addition of the vehicles, a syringe needle was inserted through the side arm into the receptor solution and 300 μL was withdrawn. The volume removed was replaced by an equal volume of fresh saline. The solution effect was accounted for in the drug flux calculations.

Example 4

Chromatographic Analysis of Oligomer

A. 14-Mer

The 14-mer (neutral methylphosphonate Oligomer having only methylphosphonate internucleosidyl linkages) and 14-mer-IA (methylphosphonate Oligomer having an internal anionic internucleosidyl linkage) were measured in the receptor solution by HPLC. These analyses were performed on a Waters 840 system consisting of two model 510 pumps, a model 481 UV detector, a model 710B WISP (sample processor), and a Digital computer model 350 microprocessor/programmer. The column used to separate the 14-mer was a 3.9 mm x 15 cm 4 μm, Waters Nova-Pak C18. A gradient elution was performed as follows for the 14-mer:

Time (min)	%A	%B
0	100	0
4	70	30
10	55	45
11	55	45
12	5	95
15	5	95
17	100	0
22	100	0

Flow rate = 1.1 mL/min, wavelength = 260 nm, retention time = 9.6 min.

A = 0.05 M TEAA, pH 7.6

B = acetonitrile/A (75:25)

B. 14-Mer-IA

The HPLC conditions were altered somewhat for measurement of the 14-mer-IA. Again, a gradient elution profile was used as described below.

Time (min)	%A	%B
0	0	100
5	28	72
14	45	55
14.2	45	55
15.5	98	2
20	98	2
22	0	100
28	0	100

Flow rate = 1.1 mL/min, wavelength = 260 nm, retention time = 10.2 min.

A = Acetonitrile/B (75:25)

B = 0.05 M ammonium acetate, pH 7.4

A chromatogram of the 14-mer-IA is shown in Figure 9 using the elution profile outlined above.

Example 5

Tissue Level Measurements of Oligomer Retained in Skin

Preliminary work was performed to determine the amount of 14-mer oligomer retained in the skin samples at the conclusion of the permeability experiments. The skins were rinsed with a small amount of water for several seconds, followed by washing for about 10 seconds with a small amount of acetonitrile to remove solid drug from the surface of the skin. The skins were then rinsed for several seconds with water. The skins were then frozen until ready for analysis (up to several weeks). The skins were thawed and the region not exposed to the donor vehicle was cut away and discarded. The hydrated skin samples were weighed and then homogenized in 0.01 M sodium phosphate, pH 7.4 using a Polytron Homogenizer for approximately 2 minutes. The homogenate was then centrifuged at 8,000 g for 15 minutes at room temperature. The supernatant was removed and analyzed directly by HPLC analysis (see below for conditions).

The chromatographic conditions were similar to those described above for the 14-mer with some minor changes noted below.

Time (min)	%A	%B
0	100	0
5	70	30
14	55	45
15.5	2	98
32	2	98
35	100	0
42	100	0

Flow rate = I.1 mL/min, wavelength = 260 nm, retention time = 11.1 min.

A = 0.05 M ammonium acetate, pH 7.0

B = acetonitrile/A (75:25)

A copy of a chromatogram obtained from a homogenized skin sample is found in Figure 10. Presence of the oligomer in the tissue homogenates was confirmed by spiking the samples with 40 μL of a 1.8 μg/mL solution of 14-mer.

Resuspension of the pellet obtained after centrifugation, followed by homogenization and recentrifugation, led to release of between 1 to 3% of the total 14-mer recovered from the original sample. These results indicate that the 14-mer was efficiently in the first extraction step.

Example 6

Measurement of Flux and Retention of Oligomers in Human Skin

Human skin which had been dermatomed to a thickness of about 5-200 μm was used. The skin was mounted in a closed glass Franz diffusion cell (as described in Example 3).

Vehicle containing oligomer and optionally enhancer (100 $\mu L/cm^2$) was placed on the surface of the skin (2 exposed surface cm^2).

The amount of oligomer diffusing through and remaining in the skin was measured by HPLC. (See Example 3)

Results are summarized in Table III. Ethanol alone was found to be an effective penetration enhancer. Addition of DMS (decylmethylsulfoxide) to ethanol generally increased the penetration rate (and cumulative amount, i.e. amount penetrated over 24 hour period) of the 6-, 10- and 14-mers through human skin relative to that from ethanol alone. Addition of water to the ethanol/DMS vehicle increased the flux (and cumulative amount) still further for the 6-mer; however, flux (and cumulative amount) for the 10-mer and 14-mer was reduced.

Addition of DMS to propylene glycol increased the flux (and cumulative amount) of the 6-mer through human skin; however, the flux (and cumulative amount) was still an order of magnitude lower compared with the ethanol/DMS vehicle. Removing the stratum corneum from human skin led to a large increase in flux (and cumulative amount) of the 6-mer, although the increase was not as dramatic as that observed with hairless mouse skin.

In comparing the cumulative amount data from hairless mouse skin with human skin for the 10-mer and the 14-mer, the cumulative amount was greater in hairless mouse skin, but was generally within an order of magnitude.

Overall, an inverse relationship of permeation rate on molecular weight was observed (i.e., the higher the molecular weight, the lower the cumulative amount).

Generally, the highest retention of oligomer both in the viable tissues (dermal layer) and stratum corneum was observed from the ethanol/water/DMS vehicle. The ratio of retained oligomer in stratum corneum to dermis was about 10-30 (Note: Since there was considerably more viable tissue than stratum corneum, the majority of oligomer retained was in the dermis). Tape stripping (to remove stratum corneum) of skin did not lead to a larger amount of 6-mer being retained in dermis as compared to retention in dermis using whole skin.

Table IV reports retention of 14-mer in dermis versus strateum corneum after treatment with 14-mer in various vehicle/enhancer combinations. Stratum corneum and dermis were separated by microwave treatment as described by Kumar et al. (Pharm. Res. 6:740-741 (1989)).

TABLE I

Permeability of Oligomers in Hairless Mouse (HM) and Human Skin (HS)

Skin	Oligomer	Donor Vehicle[a]	Cumulative Amount at 24 h ($\mu g/cm^2$)
HM	14-mer	H_2O	0.75
		EtOH	0.28
		EtOH/DMS (95:5)	5.5
		EtOH/DMS (97.5:2.5)	4.4
		EtOH/OA (95:5)[b]	0.30
		EtOH/OA (97.5:2.5)	0.24
		EtAc[c]	1.2
		EtAc/DMS (95:5)	1.1
		EtAc4/OA (95:5)	0.60
		EtOH[d]	187
		EtOH/DMS (95:5)[d]	186
		EtOH/H_2O/DMS (80:15:5)	3.5
		EtOH/H_2O/DMS (80:15:5)[e]	2.7
		EtOH/H_2O/DMS (80:15:5)[f]	2.1
		EtOH/H_2O/DMS (80:15:05)[g]	0.23
HM	14-mer-IA	H_2O	0
		EtOH	0
		EtOH/DMS (95:5)	0.61
HS	14-mer	EtOH	0.26
		EtOH/DMS (95:5)	0.24
		EtOH/OA (95:5)	0.30
		EtOH/H_2O/DMS (80:15:5)[h]	0.23

[a]Unless stated in the table footnotes, all the donor vehicles were saturated with oligomer

[b]OA = oleic acid

[c]EtAc = ethylacetate

[d]These skins were free of stratum corneum, which was removed by tape stripping.

[e]14-mer concentration in the vehicle was 1.0 mg/mL (below saturation)

[f]14-mer concentration in the vehicle was 0.5 mg/mL (below saturation)

[g]14-mer concentration in the vehicle was 0.05 mg/mL (below saturation)

[h]14-mer concentration in the vehicle was 1.0 mg/mL (below saturation)

TABLE II

A. Amounts of 14-mer Recovered from Skin Samples

Skin	Donor Vehicle	μg/gm[a]	μM[b]
HM	EtOH/DMS (95:5)	30.2	7.1
	EtOH/DMS (95:5)[c]	112	26.3
	EtOH/H_2O/DMS (80:15:5)	77	17.9
	EtOH/H_2O/DMS (80:15:5)[d]	18.4	4.3
HS	EtOH/DMS (95:5)	67.1	15.7

[a]Total μg of 14-mer recovered from the homogenized skin sample corrected for loss of 14-mer during homogenization and centrifugation (see Example 4); the gm is the wet weight of the skin as measured prior to homogenization

[b] μM concentration of 14-mer in the skin were obtained from the molecular weight of the 14-mer and the assumed density of 1.0 for the skin sample (i.e., 1.0 gm of skin is equal to 1.0 cc of skin)

[c]The HM skin used in this experiment was stripped to remove the stratum corneum

[d]The concentration of 14-mer in this vehicle was 0.5 mg/mL compared to all the other experimental vehicles, which were saturated with excess solid 14-mer

B. Retention of 14-mer in Whole Skin and Viable Tissues[a]

Skin	Section	Donor Vehicle	μg/gm[b]	μM[c]
HM	Whole	EtOH/H_2O/DMS (80:15:5)	63.2	14.8
	Viable[d]	EtOH/H_2O/DMS (80:15:5)	35.2	8.2

HS	Whole	EtOH/H_2O/DMS (80:15:5)	105.9	24.7
	Viable[d]	EtOH/H_2O/DMS (80:15:5)	7.0	1.6

[a]The weighed skin samples (hydrated) were either homogenized whole or the stratum corneum was removed, and the 14-mer content of the remaining tissue (viable epidermis and dermis) was determined. In each case, n = 2.

[b]Total μg of 14-mer recovered from the homogenized skin sample corrected for loss of 14-mer during homogenization and centrifugation (see Example 4); the gm is the wet weight of the skin as measured prior to homogenization

[c]μM concentration of 14-mer in the skin were obtained from the molecular weight of the 14-mer and the assumed density of 1.0 for the skin sample (i.e., 1.0 gm of skin is equal to 1.0 cc of skin)

[d]The viable tissue is the tissue after the stratum corneum has been removed by microwave treatment (Kumar, et al., Pharm. Res. 6:740-741 (1989)). It is a combination of the viable epidermis and the dermis.

TABLE III

Penetration of Oligomers Through Skin

A. Human Skin

Vehicle/ Enhancer	Ratio of Components	24Hr Cumulative Values: nmoles/cm2 Mean and SD		
		6 Mer	10 Mer	14 Mer
EtOH/H_2O/DMS	(80:15:5)	13.8(5.7) 2.2(2.0)	0.94(1.3)	0.18(0.17)
EtOH/DMS	(95:5)	8.2(5.4)	6.0(4.2)	0.83(1.0)
EtOH	(100:0)	3.4(2.8)	4.0(6.7)	0.37(0.48)
PG	(100:0)	0.21(0.37)	No Data	No Data
PG/DMS	(95:5)	0.57(0.50)	No Data	No Data
EtOH/DMS Tape Stripped	(95:5)	34.0(4.8)	No Data	No Data

The second value for the 6Mer came from a time study using a different skin donor, otherwise the data for the first three enhancers came from the same donor.

The data for the last three enhancers came from the same experiment but from a different donor.

B. Hairless Mouse

Vehicle/ Enchancer	Ratio of Components	24Hr Cumulative Values: nmoles/cm2 Mean and SD		
		6 Mer	10 Mer	14 Mer
EtOH/H_2O/DMS	(80:15:5)	No Data	2.08(0.82)	0.82
EtOH/DMS	(95:5)	No Data	1.94(0.22)	1.28
EtOH	(100%)	No Data	0.35(0.10)	0.065

TABLE IV
Oligomers in Human Skin t=24 Hours

Mer #	Vehicle/Enhancer	Ratio of Components	Dermis uM in Wet Skin Mean (S.D.)	Stratum Corneum uM in Wet Skin Mean (S.D.)	Ratio of SC:Dermis
6 Mer	EtOH/H_2O/DMS	(80:15:5)	66 (78)	1020 (1330)	13.8 (2.6)
	EtOH/DMS	(95:5)	12 (8)	190 (30)	28.6 (29.8)
	EtOH	(100:0)	6 (1)	88 (65)	13.4 (9.1)
10 Mer	EtOH/H_2O/DMS	(80:15:5)	3 (2)	72 (39)	23.8 (11.6)
	EtOH/DMS	(95:5)	11 (2)	360 (280)	30.3 (18.6)
	EtOH	(100:0)	6 (4)	780 (1190)	236 (376)
14 Mer	EtOH/H_2O/DMS	(80:15:5)	86 (1 Value)	70 (78)	18.6
	EtOH/DMS	(95:5)	6 (2)	150 (120)	22.7 (14.9)
	EtOH	(100:0)	5 (4)	160 (180)	62.8 (92.2)
6 Mer	EtOH/DMS (TS) [b]	(95:5)	73 (14)	Tape Stripped	Tape Stripped
	PG	(100:0)	6 (6)	47 (10)	13.1 (10.5)
	PG/DMS	(95:5)	6 (3)	138 (4)	24.3 (11.1)
6 Mer	EtOH/H_2O/DMS	(80:15:5)	Mean (A.D.)[a]	Mean (A.D.)[a]	Mean (A.D.)[a]
	30 minutes		8 (1 Value)	6 (1 Value)	8.5 (1 Value)
	1 hour		7 (0.5)	60 (10)	9.3 (0.7)
	3 hour		9 (0.9)	120 (20)	12.6 (0.6)
	8 hour		6 (3)	130 (40)	41.3 (6.1)
	24 hour		16 (7)	1360 (1040)	59.2 (8.6)
6 Mer	EtOH/Azone	(95:5)	2 (2)	10 (2)	6.2 (2.8)
	EtOH/GMO	(95:5)	4 (1)	9 (1)	2.5 (0.9)
	EtOH/H_2O/GMO	(80:15:5)	6 (1)	48 (5)	7.8 (0.9)

With the exception of the time study data for the 6Mer, the values in this table are the means of three values from three separate cells, with the SD in parenthesis.
The 6 Mer time study had one cell for the 30 minute point and 2 cells for the other time points.
[a]A.D. is the average deviation.
[b]TS=tape stripped skin (stratum corneum removed.)

Claims

1. A method of preventing or interfering with expression of a specific nucleic acid target sequence which comprises administration across skin or mucous membrane tissue of a neutral Oligomer which is complementary to and which can bind to or interact with said specific nucleic acid sequence or a modulating nucleic acid sequence which modulates expression of the target sequence.

2. A method according to claim 1 wherein said neutral Oligomer comprises an alkyl- or aryl-phosphonate Oligomer, a phosphotriester Oligomer, a phosphoramidate Oligomer, a carbamate Oligomer, a sulfamate Oligomer, a morpholino Oligomer, an alkyl or aryl- phosphonothioate Oligomer, or a formacetal Oligomer.

3. A method according to claim 2 wherein said neutral Oligomer comprises a neutral methylphosphonate Oligomer.

4. A method according to claim 3 wherein said neutral Oligomer is applied in a formulation which comprises a short chain aliphatic alcohol.

5. A method according to claim 1 wherein said nucleic acid target sequence codes for a mediator of inflammation.

6. A method according to claim 5 wherein said mediator is selected from a cytokine, a growth factor, a cell adhesion molecule or a receptor thereof.

7. A method according to claim 1 wherein said target sequence comprises an RNA region which codes for an initiation codon region, a polyadenylation region, a mRNA cap site, or a splice junction.

8. A method according to claim 1 wherein said target sequence comprises a region of the gene which codes for renin or its mRNA transcript.

9. A method according to claim 8 wherein said target sequence comprises an initiator codon region, a polyadenylation region, a mRNA cap site or a splice junction.

10. A method according to any of claims 1 or 2 wherein said Oligomer comprises a conjugation partner.

11. A method according to claim 10 wherein said conjugation partner is selected from intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents, cross-linking agents, hydrolytic or nucleolytic agents, prochelates, or DNA modifying groups.

12. A method of delivery of an antisense oligomer to skin or mucous membrane tissue which prevents or interferes with expression of a specific nucleic acid target sequence which comprises application of a formulation which comprises a neutral Oligomer which is complementary to and which can bind to or interact with said nucleic acid sequence or a modulating nucleic acid sequence which alter expression of the target sequence.

13. A method according to claim 12 wherein said neutral oligomer comprises an alkyl- or aryl-phosphonate Oligomer, a phosphotriester Oligomer, a phosphoramidate Oligomer, a carbamate Oligomer, a sulfamate Oligomer, a morpholino Oligomer, an alkyl or aryl-phosphonothioate Oligomer or a formacetal Oligomer.

14. A method according to claim 13 wherein said neutral Oligomer comprises a methylphosphonate oligomer.

15. A method according to claim 14 wherein said formulation comprises a short chain aliphatic alcohol.

16. A method according to claim 15 wherein said formulation further comprises a flux enhancer.

17. A method according to claim 12 or 13 wherein said Oligomer comprises a conjugation partner.

18. A method according to claim 17 wherein said conjugation partner is selected from intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents, cross-linking agents, hydrolytic or nucleolytic agents, prochelates, or DNA modifying groups.

19. A neutral Oligomer which is capable of penetrating skin or mucous membranes and which is capable of selectively binding to or interacting with a specific nucleic acid target sequence.

20. An Oligomer according to claim 19 wherein said target sequence comprises an RNA region which codes for an initiator codon region, a polyadenylation region, a mRNA cap site or a splice junction or a DNA region which results in alteration of gene expression.

21. An Oligomer according to claim 20 which comprises an alkyl- or aryl-phosphonate Oligomer, a phosphotriester Oligomer, a phosphoramidate Oligomer, a carbamate Oligomer, a sulfamate Oligomer, a morpholino Oligomer, an alkyl- or aryl-phosphonothioate Oligomer or a formacetal Oligomer.

22. An Oligomer according to claim 21 which comprises a methylphosphonate Oligomer.

23. An Oligomer according to claim 19 which comprises an alkyl- or aryl-phosphonate Oligomer, a phosphotriester Oligomer, a phosphoramidate Oligomer, a carbamate Oligomer, a sulfamate Oligomer, a morpholino Oligomer, an alkyl- or aryl-phosphonothioate Oligomer, or a formacetal Oligomer.

24. An Oligomer according to claim 23 which comprises a methylphosphonate Oligomer.

25. An Oligomer according to claim 19 wherein said nucleic acid target sequence comprises a region of the gene which codes for renin or its mRNA transcript or a region which results in alteration of renin expression.

26. An Oligomer according to claim 25 wherein said target sequence comprises an initiator codon region, a polyadenylation region, or a mRNA cap site or a splice junction.

27. An Oligomer according to any of claims 19, 20 or 25 which comprises a conjugation partner.

28. An Oligomer according to claim 27 wherein said conjugation partner is selected from intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents, cross-linking agents, hydrolytic or nucleolytic agents, pro-chelates, or DNA modifying groups.

29. A method of delivering an Oligomer to an animal for therapeutic purposes which comprises administration of a neutral Oligomer across skin or mucous membranes.

30. A method according to claim 29 wherein said Oligomer is administered transdermally.

31. A method according to claim 29 wherein said Oligomer is administered to mucous membranes.

32. A method according to claim 31 wherein said Oligomer is administered by inhalation.

33. A method according to claim 29 wherein said Oligomer prevents or interferes with expression or function of a mediator of inflammation.

34. A method according to claim 29 wherein said Oligomer comprises an alkyl- or aryl-phosphonate Oligomer, a phosphotriester Oligomer, a carbamate Oligomer, a sulfamate Oligomer, a morpholino Oligomer, an alkyl- or aryl phosphonothioate Oligomer or a formacetal Oligomer.

35. A method according to claim 34 wherein said Oligomer is a methylphosphonate Oligomer.

36. A method according to claim 35 wherein all internucleosidyl linkages of said Oligomer are methylphosphonate internucleosidyl linkages.

37. A method according to any of claims 29, 34, 35 or 36 wherein said Oligomer comprises a conjugation partner.

38. A method according to claim 37 wherein said conjugation partner is selected from intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents, cross-linking agents, hydrolytic or nucleolytic agents, prochelates or DNA modifying groups.

39. A method according to claim 29 wherein said Oligomer prevents or interferes with expression or function of a mediator of cell adhesion.

40. A method according to claim 29 wherein said Oligomer prevents or interferes with an agent which mediates cell proliferation.

41. A method according to claim 29 wherein said Oligomer prevents or interferes with cancer cell proliferation.

42. A method according to claim 29 wherein said target sequence comprises a region of the gene which codes for renin or its mRNA transcript or a region which results in alteration of renin expression.

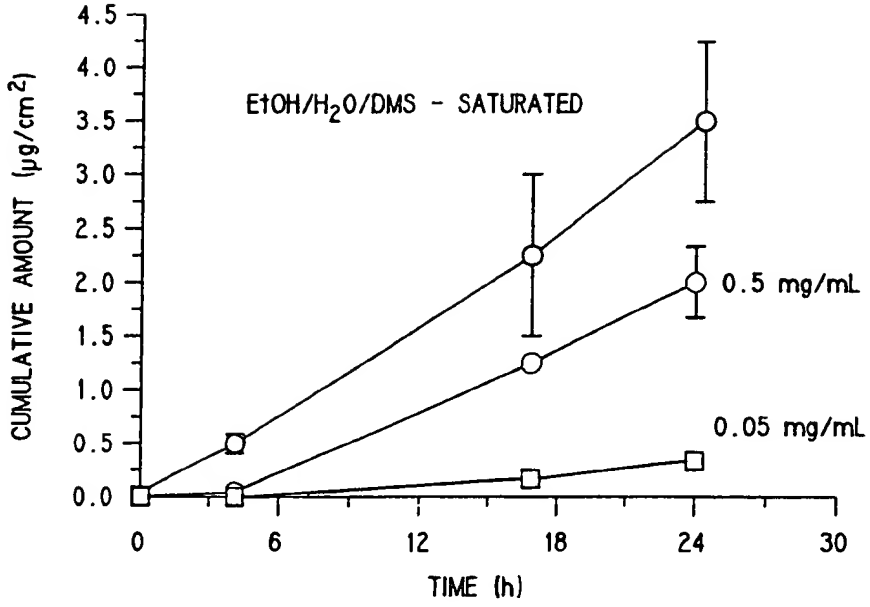
EtOH/H2O/DMS - SATURATED
0.5 mg/mL
0.05 mg/mL
CUMULATIVE AMOUNT (µg/cm2)
TIME (h)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
6
12
18
24
30

FIG. 1.

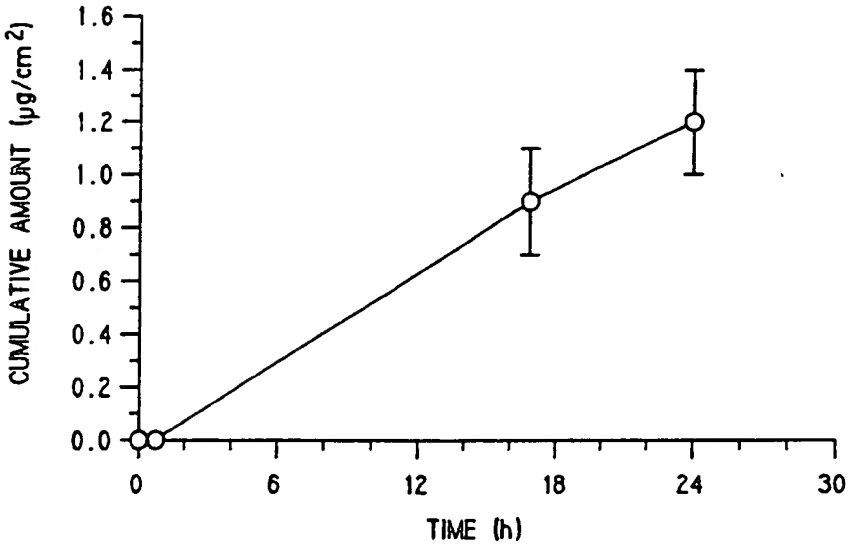
CUMULATIVE AMOUNT (µg/cm2)
TIME (h)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
30

FIG. 2.

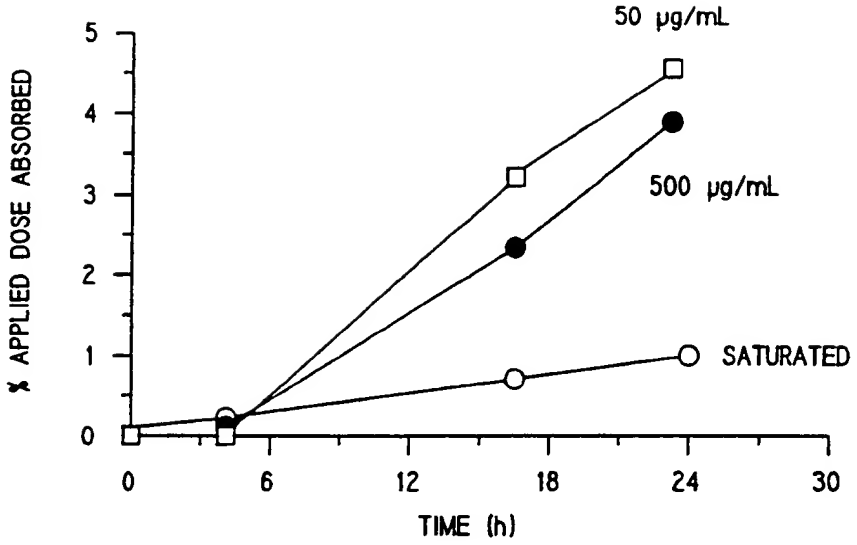
50 µg/mL
500 µg/mL
SATURATED
% APPLIED DOSE ABSORBED
TIME (h)
0
1
2
3
4
5
0
6
12
18
24
30

FIG. 3.

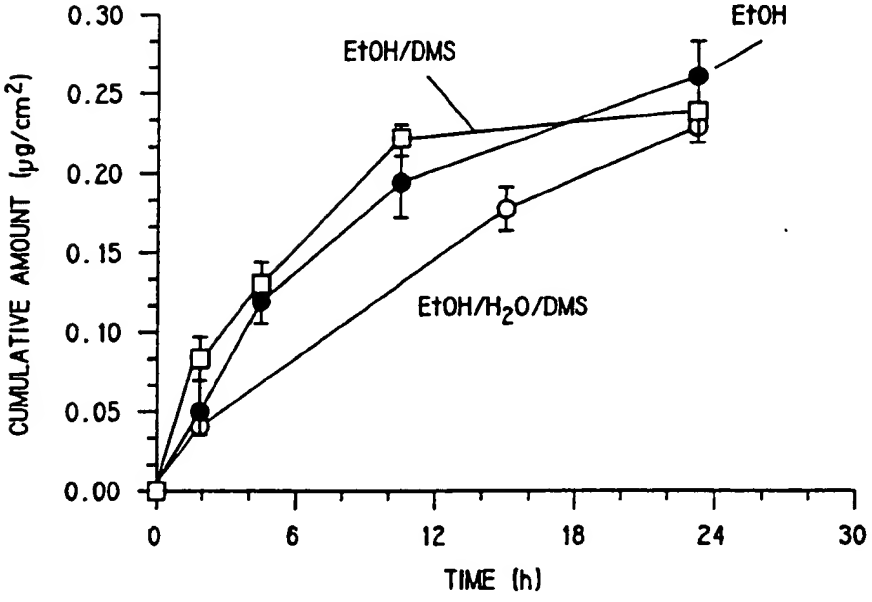
EtOH
EtOH/DMS
EtOH/H2O/DMS
CUMULATIVE AMOUNT (µg/cm2)
TIME (h)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
6
12
18
24
30

FIG. 4.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/04370

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 31/70; C07H 15/12
US CL :514/44; 536/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,689,320 (Kaji) 25 August 1987, especially col. 16, example 19.	1-42
Y	Nucleic Acids Research, Volume 15, Number 24, issued 1987, C. Cazenave et al, "Rate of Degradation of [α]- and [β]-oligodeoxy-nucleotides in Xenopus Oocytes. Implications for Anti-Messenger Strategies", pages 10507- 10521, entire document.	1-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 11 August 1992	Date of mailing of the international search report 28 AUG 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DEBORAH CROUCH, PH.D. Telephone No. (703) 308-1126

Form PCT/ISA/210 (second sheet)(July 1992)*